POINTS TO CONSIDER

SCIENTIFIC ABSTRACT

Improvements in radiochemotherapy have correspondingly improved the prognosis of patients with EBV+ve Hodgkin and non-Hodgkin lymphoma. However, patients resistant to the standard therapeutic approaches have a poor outcome. Moreover, life expectancy and quality of life of patients cured of lymphoma are both significantly reduced by treatment related mortality and morbidity. These limitations of current treatment protocols illustrate the need for more effective and less toxic therapeutic approaches. In Hodgkin's and non-Hodgkin's lymphomas up to 40% of specimens have been shown to carry EBV-DNA and express EBV-genes. Our group has successfully generated EBV-specific CTL (EBV-CTL) in patients with EBV +ve Hodgkin's lymphoma. After infusion these CTL home to the tumor sites and persist in the circulation for up to 9 months. However although transient clinical benefits were seen, no complete responses were acheived with CTL therapy alone.

There are two possible reasons for the lack of persistence and efficacy of the CTL in vivo. Firstly, the EBV-CTL may lack clinically significant tumor specificity. When lymphoblastoid cell lines (LCL) are used as EBV-antigen presenting cells (APC) as in the original EBV-CTL protocol, the CTL populations that are activated are preferentially directed against immunodominant EBV-proteins. These immunogenic proteins are not expressed in EBV+ve lymphomas arising in the immunocompetent individual. Instead, the EBV-antigens on the lymphoma cells are restricted to the expression of a subset of latent proteins, EBNA1, LMP1, LMP2A and BARF0.[1] LCL have limited efficacy in stimulating CTL directed against these subdominant proteins. LMP2A epitopes were shown to be conserved among EBV+ve lymphoma biopsy samples, displaying little heterogeneity between viral strains. Also most donors have a low but measurable frequency of circulating LMP2A-specific CTL that can be activated and expanded in vitro. Hence LMP2A may be the protein of choice to be targeted by CTL in patients with EBV+ve lymphoma.

A promising strategy to stimulate LMP2A-specific CTL (LMP2-CTL) is the genetic modification of DC that direct the CTL response to the virally transduced genes, followed by expansion of the LMP2-CTL population using LCL which have been genetically modified to over-express LMP2.[2] [3,4]This approach allows expression of the whole protein leading to presentation of multiple, undefined antigen epitopes. In a RAC reviewed and FDA-approved protocol, we are already using a recombinant adenovirus encoding LMP2A for transduction of DC and LCL. These genetically modified DC and LCL are used as APCs to generate and expand LMP2-CTL *in vitro*.There is a a second reason for a lack of persistence of infused CTL *in vivo*. The size of the T cell compartment is maintained at a steady state by a number of potent homeostatic mechanisms. We believe that the failure of infused EBV-CTL to persist may be in part due to failure of the infused CTL to undergo adequate expansion *in vivo*.[5] Since these homeostatic mechanisms drive lymphoproliferation during lymphopenia, we now propose to extend our original RAC reviewed and FDA approved protocol to deplete the patient's lymphoid compartment prior to LMP2-specific CTL infusion. In addition, lymphoid depletion will also result in a loss of the inhibitory T cells such as the CD4+CD25+ negative regulatory cells which infiltrate tumors such as seen in Hodgkin Disease. For this purpose, we will use short-lived CD45 monoclonal antibodies (MAbs), which we have shown can profoundly deplete lymphocytes in peripheral blood and lymphoid organs, whilst sparing hematopoietic progenitor cells.[6] Subsequent adoptive transfer of LMP2-CTL should result in expansion of the infused cells to restore the T cell compartment In the phase I study proposed here, patients will receive a fixed dose of CD45 Mabs followed by escalating doses of autologous LMP2-CTL will be administered to Lymphoma patients with refractory or relapsed disease.

Of note the final cellular product administered to the patient (LMP-2 specific CTL) and the viral vector used to generate them (Ad5/35-LMP2) are identical to the materials used in an earlier RAC-reviewed FDA approved protocol (RAC Protocol 0105-473).